EXAMINER'S AMENDMENT

1. The Office communication mailed October 31, 2008, has been vacated in favor of entering the following amendment.

2. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

3. Authorization for this examiner's amendment was given in a telephone interview with Carol Johns on October 15, 2008.

4. The application has been amended as follows:

In the specification:

The paragraph at page 5, beginning in line 27, has been replaced with the following:

On the other hand, in a preferred embodiment, the PCOTH polypeptide consists of a putative 100 amino acid sequence set forth in SEQ ID NO: 2 (GenBank® Accession No. AB 113650). PCOTH is encoded by the open reading frame of SEQ ID NO: 1 and comprises a collagen triple helix repeat (FIG. 1 (C)). The present application also provides an isolated protein encoded from at least a portion of the *PCOTH* polynucleotide sequence, or polynucleotide sequences at

least 30% and more preferably at least 40% complementary to the sequence set forth in SEQ ID NO: 1 (*LOC221179*(XP_167955)).

The paragraph at page 8, beginning in line 33, has been replaced with the following:

FIG. 1 (A) depicts photographs showing the result of validation of over-expression of *D4493* (*PCOTH*) in prostate cancer cells by RT-PCR. The microdissected normal prostate duct epithelial cells (N) and prostate cancer cells (T) from the same individual were compared by semiquantitative RT-PCR. ACTB was used for normalization of the results. (B) depicts photographs showing the result of Northern blot analysis of normal human multiple tissues. High and localized expression in testis and prostate and minor expression in heart and bone marrow were detected. (C) depicts the amino acid sequence of D4493 (PCOTH) product (SEQ ID NO:2). The product consists of 100 amino acids and has collagen triple helix repeats which is characterized by the G-X-X motif repeat. G is glycine and X is ~~preferrably~~ preferably proline.

The paragraph at page 17, beginning in line 9, has been replaced with the following:

According to the present invention another gene, *PCOTH*, was also identified to be specifically over-expressed in prostate cancer cells compared to corresponding non-cancerous tissues. The identified gene was identical with *LOC221179* (XP_167955). However, the *PCOTH* gene was revealed to encode a 100-amino acid protein set forth in SEQ ID NO: 2 (GenBank® Accession No. AB113650) encoded by the open reading frame consisting of 300 nucleotides shown in SEQ ID NO: 1 which differed from that known for *LOC221179* (XP_167955). PCOTH was shown to comprise a collagen triple helix repeat and

its exogenous product was localized in the cell membrane (FIG. 1). Therefore, the gene was dubbed "*prostate collagen triple helix*".

The paragraph at page 18, beginning in line 24, has been replaced with the following:

When *E. coli* is a host cell and the vector is amplified and produced in a large amount in *E. coli* (e.g., JM109, DH5α, HB101 or XL1Blue), the vector should have "ori" to be amplified in *E. coli* and a marker gene for selecting transformed *E. coli* (e.g., a drug-resistance gene selected by a: drug such as ampicillin, tetracycline, kanamycin, chloramphenieol or the like). For example, M13-series vectors, pUC-series vectors, pBR322, pBluescript, pCR-Script, etc. can be used. In addition, pGEM-T, pDIRECT and pT7 can also be used for subcloning and extracting eDNA as well as the vectors described above. When a vector is used to produce the protein of the present invention, an expression vector is especially useful. For example, an expression vector to be expressed in *E. coli* should have the above characteristics to be amplified in *E. coli.* When *E. coli,* such as JM109, DH5α, HB101 or XL1 Blue, are used as a host cell, the vector should have a promoter, for example, lacZ promoter (Ward et al., Nature 341:544-6 (1989); FASEB J 6:2422-7 (1992)), araB promoter (Better et al., Science 240:1041-3 (1988)), T7 promoter or the like, that can efficiently express the desired gene in E. coli. In that respect, pGEX-5X-1 (Pharmacia), "QIAexpress™ system" (Qiagen), pEGFP and pET (in this case, the host is preferably BL21 which expresses T7 RNA polymerase), for example, can be used instead of the above vectors. Additionally, the vector may also contain a signal sequence for polypeptide secretion. An exemplary signal sequence that directs the polypeptide to be secreted to the periplasm of the E. coli is the pelB signal sequence (Lei et al., J Bacteriol 169:4379 (1987)). Means for introducing of the vectors into the target host cells include, for example, the calcium chloride method, and the electroporation method.

The paragraph at page 24, beginning in line 23, has been replaced with the following:

For example, measurement of absorbance, enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA) and/or immunofluorescence maybe used to measure the antigen binding activity of the antibody of the invention. In ELISA, the antibody of the present invention is immobilized on a plate, a polypeptide of the invention is applied to the plate, and then a sample containing a desired antibody, such as culture supernatant of antibody producing cells or purified antibodies, is applied. Then, a secondary antibody that recognizes the primary antibody and is labeled with an enzyme, such as alkaline phosphatase, is applied, and the plate is incubated. Next, after washing, an enzyme substrate, such as p- nitrophenyl phosphate, is added to the plate, and the absorbance is measured to evaluate the antigen binding activity of the sample. A fragment of the polypeptide, such as a C-terminal or N- terminal fragment, may be used as the antigen to evaluate the binding activity of the antibody. BIAcore™ (Pharmacia) may be used to evaluate the activity of the antibody according to the present invention.

The paragraph at page 26, beginning in line 19, has been replaced with the following:

The nucleotide sequence of siRNAs may be designed using an siRNA design 20 computer program available from the Ambion website on the world wide web ~~(http://www.ambion.com/techlib/misc/siRNA_finder.html)~~. Nucleotide sequences for tile siRNA are selected by the computer program based on the following protocol:

Selection of siRNA Target Sites:

1. Beginning with the AUG start codon of the object transcript, scan downstream for AA dinucleotide sequences. Record the occurrence of each AA

and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl, et al. recommend against designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75 bases) as these may be richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with the binding of the siRNA endonuclease complex.

2. Compare the potential target sites to the human genome database and eliminate from consideration any target sequences with significant homology to other coding sequences. The homology search can be performed using BLAST, which can be found on the NCBI server on the world wide web ~~at: www.nebi.nlm.nih.gov/BLAST/~~.

3. Select qualifying target sequences for synthesis. At Ambion, preferably several target sequences can be selected along the length of the gene for evaluation.

The paragraph at page 32, beginning in line 32, has been replaced with the following:

Alternatively, in another embodiment of the screening method of the present invention, a two-hybrid system utilizing ceils may be used ("MATCHMAKER Two-Hybrid system", "Mammalian MATCHMAKER Two-Hybrid Assay Kit", i "MATCHMAKER one-Hybrid system" (Clontech); "HybriZAP™ Two-Hybrid Vector System" (Stratagene); the references "Dalton and Treisman, Cell 68:597-612 (1992)", "Fields and Stemglanz, Trends Genet 10:286-92 (1994)").

The paragraph at page 33, beginning in line 25, has been replaced with the following:

A biosensor using the surface plasmon resonance phenomenon may be used as a mean for detecting or quantifying the bound compound in the present

invention. When such a biosensor is used, the interaction between the polypeptide of the invention and a test compound can be observed real-time as a surface plasmon resonance signal, using only a minute amount of polypeptide and without labeling (for example, BIAcore™, Pharmacia). Therefore, it is possible to evaluate the binding between the polypeptide of the invention and a test compound using a biosensor such as BIAcore™.

The paragraph at page 37, beginning in line 2, has been replaced with the following:

In the present invention, a biosensor using the surface plasmon resonance phenomenon may be used as a mean for detecting or quantifying the bound protein. When such a biosensor is used, the interaction between the proteins can be observed real-time as a surface plasmon resonance signal, using only a minute amount of polypeptide and without labeling (for example, BIAcore™, Pharmacia). Therefore, it is possible to evaluate the binding between the MICAL2- PV polypeptide and actin using a biosensor such as BIAcore™.

The paragraph at page 38, beginning in line 2, has been replaced with the following:

Alternatively, in another embodiment of the screening method of the present invention, a two-hybrid system utilizing ceils may be used ("MATCHMAKER Two-Hybrid system", "Mammalian MATCHMAKER Two-Hybrid Assay Kit", i "MATCHMAKER one-Hybrid system" (Clontech); "HybriZAP™ Two-Hybrid Vector System" (Stratagene); the references "Dalton and Treisman, Cell 68:597-612 (1992)", "Fields and Stemglanz, Trends Genet 10:286-92 (1994)").

The paragraph at page 48, beginning in line 25, has been replaced with the following:

Total RNA was extracted from cultured cells and clinical samples using TRIzol™ Reagent (Invitrogen) according to the manufacturer's protocol. Extracted RNA was treated with DNase I (Roche) and reversely transcribed for single-stranded eDNAs using oligo(dT)l6 primer with Superscript™ II reverse transcriptase (Roche). Appropriate dilutions of each single-stranded eDNA were prepared for subsequent PCR amplification by monitoring the 13-actin (ACTB) as a quantitative control. The primer sequences were

5'-CATCCACGAAACTACCTTCAACT-3' (SEQ.ID.NO. 11) and 5'-TCTCCTTAGAGAGAAGTGGGGTG-3' (SEQ.ID.NO. 12) for ACTB;

5'-CCGACACTCTGGGTAGGAGA-3' (SEQ.ID.NO. 13) and 5'-TACGTGAGCTCTGAGGACCA-3' (SEQ.ID.NO.14) for D4493; and

5'-GCAGGGATATCTTTGAGAAA-3' (SEQ.ID.NO. 15) and 5'-CCAGGATCTGCACAAATACA-3' (SEQ.ID.NO. 16) for A5736. All reactions involved initial denaturation at 94°C for 2 min followed by 21 cycles (for ACTB) or 35 cycles (for D4493 and A5736) at 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min, on a GeneAmp™ PCR system 9700 (PE Applied Biosystems).

The paragraph at page 49, beginning in line 18, has been replaced with the following:

COS7 cells were transfected transiently with pCAGGS neo-D4493 and pcDNA3.1 (-)-A5736-myc-His using FuGENE™ 6 (Roche) according to manufacture's instruction, then were fixed with 4% paraformaldehyde and permeablilized with 0.2% Triton X-100in PBS for 3min at room temperature. Next, the cells were covered with blocking solution (3% BSA/PBS containing 0.2% Triton X-100) for 30min at room temperature, and incubated with a rat anti-HA antibody (Roche) or a rat anti-myc antibody (Sigma) in blocking solution for 60 min at room temperature. After washing with PBS, cells were stained by a FITC-conjugated anti-rat secondary antibody (Organon teknika), and

Rhodamine-conjugated anti-mouse secondary antibody (ICN Biomedicals) for 60 min at room temperature. Specimen was mounted with VECTASHIELD (VECTOR Laboratories, Inc, Burlingame, CA) containing 4',6'-diamidine-2'-phenylindolendihydrochrolide (DAPI) and visualized with Spectral Confocal Scanning Systems (Leica).

In the claims:

Claim 1. (Currently Amended) A substantially pure polypeptide ~~selected from the group consisting of: (a) a polypeptide~~ comprising the entirety of the amino acid sequence of SEQ ID NO: 4~~; (b) a polypeptide that comprises the amino acid sequence of SEQ ID NO: 4 in which ten or fewer amino acids are substituted, deleted, inserted, and/or added and that binds actin and induces cell proliferation in a manner equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 4; and (c) a polypeptide comprising amino acids 1-737 and 759-976 of SEQ ID NO: 4~~.

Claims 2-5. (Canceled)

Claim 6. (Previously Presented) A method for producing the polypeptide of claim 1, said method comprising the steps of:

(a) culturing a host cell comprising:

(1) a polynucleotide encoding the polypeptide; or

(2) a vector comprising the polynucleotide encoding the polypeptide;

(b) allowing the host cell to express the polypeptide; and

(c) collecting the expressed polypeptide.

Claims 7-33. (Canceled)

Conclusion

4. Claims 1 and 6 have been allowed.

5. Claim 6 has been renumbered as claim 2.

6. The art made of record is considered pertinent to Applicant's disclosure. Ashida et al. (*Cancer Res.* 2004 Sep 1; **64** (17): 5963-5972) teaches molecular features of the transition from prostatic intraepithelial neoplasia (PIN) to prostate cancer: genome-wide gene-expression profiles of prostate cancers and PINs. Ashida et al. (*Clin. Cancer Res.* 2006 May 1; **12** (9): 2767-2773) teaches expression of novel molecules, MICAL2-PV (MICAL2 prostate cancer variants), increases with high Gleason score and prostate cancer progression

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephen L. Rawlings whose telephone number is (571) 272-0836. The examiner can normally be reached on Monday-Friday, 8:30AM-5:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Larry Helms, Ph.D. can be reached on (571) 272-0832. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Stephen L. Rawlings/

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slr
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